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Docket/Matter No. 20.US1.PCT

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:

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| Applicant | : | Cohen, et al. |
| Appl. No. | : | 09/463,075 |
| Filed | : | January 14, 2000 |
| Title | : | BIALLELIC MARKERS FOR USE IN CONSTRUCTING A HIGH DENSITY DISEQUILIBRIUM MAP OF THE HUMAN GENOME |

Grp./A.U. : 1655

Examiner : B. Sisson



Honorable Commissioner of Patents
Washington, D.C. 20231

**AMENDMENT AND RESPONSE**

Sir:

In response to the Office Action dated May 22, 2001, in the above-referenced U.S. patent application, Applicants respectfully request reconsideration in view of the following remarks.

***The invention***

The present invention provides novel methods for generating high resolution maps of the human genome or portions thereof. Specifically, the methods involve obtaining a human nucleic acid library comprising genomic DNA fragments covering at least 100 kb of the human genome, ordering the fragments, sequencing selected regions of a plurality of the fragments, and identifying single nucleotide polymorphisms (SNPs) within the sequenced regions, wherein the SNPs have a given heterozygosity rate and inter-marker spacing (e.g. a heterozygosity rate of 0.18 and a spacing of less than 50 kb in independant claim 86).

As discussed in the present specification, these methods represent an important advance over previous methods in that they allow the systematic identification of evenly

spaced, highly heterozygous, frequent markers throughout the genome. Such collections of markers are essential for performing many important types of genetic analyses. As described on page 15, lines 9-11, of the specification,

> The even distribution of markers along the chromosomes is critical to the future success of genetic analyses. In particular, a high density map having appropriately spaced markers is essential for conducting association studies on sporadic cases, aiming at identifying genes responsible for detectable traits....

Accordingly, the maps available using the present methods are superior to previously-existing maps, such as those based on microsatellite markers, which were mostly useful for linkage analyses. As described on page 15, lines 12-17, of the specification,

> [G]enetic studies have mostly relied in the past on a statistical approach called linkage analysis, which took advantage of microsatellite markers to study their inheritance pattern within families from which a sufficient number of individuals presented the studied trait. Because of intrinsic limitations of linkage analysis, which will be further detailed below, and because these studies necessitate the recruitment of adequate family pedigrees, they are not well suited to the genetic analysis of all traits, particularly those for which only sporadic cases are available (e.g. drug response traits), or those which have a low penetrance within the studied population.

Importantly, the present methods involve the use of SNP markers, instead of more traditional markers such as microsatellites, as those previously-used markers are not present at high enough frequencies in the genome to allow analyses such as association studies.

***Rejections under 35 U.S.C. §103***

The Examiner rejects claims 86, 88, 89 and 92-105 under 35 U.S.C. § 103 as allegedly obvious over Aguirre et al. ("Aguirre") in view of Scheffield et al. ("Scheffield"). According to the Examiner, Aguirre teaches a method of detecting single nucleotide polymorphisms in human genomic material by making a genomic library and identifying sequences using known primers or probes, and Scheffield teaches subcloning human genomic sequences into vectors such as BAC or YAC vectors, and using STSs to identify the location of particular sequences. The Examiner alleges that, in view of these two references, it would have been obvious to one of skill in the art at the time of the invention to identify SNPs in sequences identified from a genomic library using STSs. Applicants respectfully traverse this rejection.

As discussed below, in contrast to the present methods, neither of the references cited by the Examiner in the Office Action teaches the identification of sets of highly heterozygous, high density, evenly-spaced SNP markers. Accordingly, neither of the references discloses nor suggests the presently-claimed invention, either alone or in combination.

According to the Examiner, the primary reference cited by the Examiner, Aguirre, is similar to the presently claimed invention because it discloses a method for detecting SNPs in human genomic material. Specifically, the Examiner states that Aguirre teaches the identification of specific sequences from a genomic library using primers or probes, and that because the primers or probes have a known genomic localization, the selected sequences can be ordered. Further, while Aguirre allegedly describes this method for sequences smaller than 100 KB, they also teach the use of BACs and YACs (see, page 3 of the Office Action).

Applicants submit that, regardless of the Examiner's characterization of the teachings of Aguirre, this reference fails to teach the identification of sets of high density SNPs (e.g. markers with an inter-marker spacing of less than 50 kb), as required by the present claims. Specifically, Aguirre describes the identification of the genomic location of a particular disease-causing gene in dogs, as well as the identification of markers, largely microsatellite markers, in the genomic region of the gene. As described by

Aguirre, these markers are useful for linkage studies, i.e. to follow the segregation of the disease gene in dogs :

> Using the linkage test of the present invention, the DNA segments may be assessed for their ability to co-segregate with the prcd gene locus (e.g. a locus score may be calculated), and thus determine the usefulness of each DNA segment in a molecular diagnostic assay for detection of prcd or the carrier status. (see, column 6, lines 28-33 of Aguirre).

Consistent with their goal of identifying markers useful for linkage analysis, and in contrast to the present invention, Aguirre neither discloses nor suggests the generation of sets of high frequency SNPs. Indeed, there are at least two major differences between the markers presented by Aguirre in Table 1 and Figure 1 and the present markers. First, a number of the markers described by Aguirre are not SNPs, but are instead non-SNP markers such as microsatellites. As discussed supra, the use of SNPs is an essential aspect of the present invention. Second, the inter-marker distance of Aguirre's markers is vastly greater than that required by the present claims. Specifically, Aguirre states that their 16 markers (shown in Table 1 and Figure 1) cover a distance of more than 37.5 centimorgans (see, column 6, lines 39-43), making an average inter-marker distance of more than 2 centimorgans. While the relationship between map distance and physical distance can vary, in mammals a typical relationship is one megabase of DNA per centimorgan, which would indicate an inter-marker spacing of more than 2 million bases for their markers. Strikingly, this is more than ***40 times*** the spacing specified in the present claims (i.e. less than 50 kb in claim 86). Therefore, Aguirre clearly fails to disclose the inter-marker spacing recited in the present claims, and, as the number of markers provided by Aguirre are more than sufficient for the type of linkage analyses described in their patent, they would have provided no suggestion or motivation for one of skill to decrease the disclosed level of spacing.

Further, the two sections of Aguirre referred to specifically by the Examiner in the Office Action also fail to disclose or suggest the present invention. For example, the Examiner refers to column 4 of the specification in support of the statement that Aguirre

"discloses a method for the detection of single nucleotide polymorphisms in human genomic material..." In fact, column 4 of Aguirre simply provides a definition of the term " marker" in the "Definitions" section of the specification, and states that "markers" can be identified using any of a number of known techniques, including microsatellites, short tandem repeat (STR) amplification, analyses of RFLPs, SNPs, deletions, insertions, or random amplified polymorphic DNA (RAPD) analysis. In no way does this section disclose or suggest the generation of a set of high density, highly heterozygous SNPs as required by the present claims.

In addition, the Examiner refers to column 18 and states that Aguirre describes the use of BAC and YACs in their libraries. In fact, the paragraph marked by the Examiner in column 18 simply states that additional markers useful for linkage analysis can be identified in the genomic region (of over 37.5 centimorgans) known to be linked to the prcd gene locus, and that DNA in the region can be included in libraries of BAC or YAC vectors. Consistent with the rest of the specification, this section in no way discloses or suggests the selection of SNP markers in particular (as opposed to, e.g. microsatellite markers), nor the desireability of generating a collection of densely-spaced markers.

The second reference cited by the Examiner, Scheffield, also fails to disclose or suggest the present invention. According to the Examiner, Scheffield discloses subcloning human genomic sequences into BAC and YACs and using specific sequences (STSs) to identify the location of the sequences. Scheffield also allegedly teaches subcloning the BAC and YAC clones and detecting mutations within the inserts. See, page 3 of the Office Action.

Applicants respectfully submit that Scheffield in no way discloses or suggests the present invention. As discussed *infra*, Scheffield describes the cloning of a chromosomal breakpoint thought to underlie a particular human disorder, and is in no way related to the generation of sets of markers for genomic analysis, let alone sets of closely-linked SNP markers.

In the Office Action, the Examiner refers to columns 61 to 63 of Scheffield in support of the rejection of the present claims. This section describes the use of BAC and YAC vectors in the cloning of the chromosomal breakpoint thought to be responsible for

a particular disease. To do this, Scheffield identified YACs and BACs corresponding to the genomic region containing the breakpoint, subcloned particular YACs and BACs, and identified particular subclones in the immediate vicinity of the breakpoint. In this way, they were able to identify two genes in the region of the breakpoint, and by sequencing one of the genes, they identified several mutations that appear to underlie the disorder.

Applicants remind the Examiner that, as described supra, the present methods are directed to the generation of sets of highly heterozygous SNPs present at an inter-marker spacing of less than 50 kb, covering at least 100 kb of the human genome. In contrast, Scheffield describes the identification of several single nucleotide mutations within an apparent disease-causing gene, in certain affected individuals. Thus, the teachings of Scheffield differ from the present methods in several critical respects. First, the heterozygosity of the mutations identified by Scheffield is certainly lower than the 0.18 required by the present claims, as three of the four mutations were not found in 128 unaffected individuals, and a fourth was not seen in 12 other individuals. Second, Scheffield only identified mutations in a single gene, whereas the present claims require the sequencing of selected regions in a "plurality" of genomic fragments, and the identification of SNPs within the selected regions (i.e. within the regions in a plurality of fragments). In addition, this region is smaller than the 100 kb minimum genomic size required by the present claims.

Therefore, Scheffield fails to disclose the identification of a set of markers spaced at an inter-marker distance of less than 50 kb over a genomic region of 100 kb, and further fail to disclose the identification of markers having a heterozygosity of greater than 0.18, as required by the present claims.

In view of the above, Applicants respectfully submit that the references cited by the Examiner, alone or in combination, fail to disclose or suggest the presently-claimed methods. For example, neither of the references disclose or suggest the identification of sets of SNP markers having a heterozygosity rate of at least 0.18 and an inter-marker spacing of less than 50 kB over a a genomic distance of at least 100 kb. Further, as the markers described in each of these references were identified for entirely different purposes than the markers obtained using the present methods (Aguirre is directed to

markers useful for meiotic linkage mapping of a single disease causing gene, and Scheffield describes the identification of several rare mutations within a single gene), even if each of the elements had been present neither of these references would have provided any motivation or suggestion to combine or modify the elements to bring about the presently-claimed invention. Accordingly, as a *prima facie* case of obviousness requires that each element of a claim be present in the prior art, as well as a suggestion or motivation to modify or combine the teachings of the prior art (see, e.g. MPEP § 2142), a *prima facie* case has clearly not been established in the present case.

In view of all of the above, Applicants submit that the present claims are indeed non-obvious over Aguirre in view of Sheffield, and thus urge the withdrawal of the standing rejection of the claims under 35 U.S.C. § 103.

Please charge any additional fees, or credit overpayment to Deposit Account No. 50-1181.

Respectfully submitted,

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